Claims

1. A method for screening for an agent that modulates TGF-β- and/or BMP-mediated signaling, comprising the steps of:

(a) contacting

(i) a first polypeptide comprising a HECT E3 ubiquitin ligase WW domain, or a variant thereof in which the ability of the polypeptide to bind to a Smad protein is not substantially diminished relative to the HECT E3 ubiquitin ligase;

(ii) a second polypeptide comprising a Smad PY motif, or a variant thereof in which the ability of the polypeptide to bind to an E3 ubiquitin ligase is not substantially diminished relative to a native Smad protein comprising the PY motif; and

(iii) a candidate agent; under conditions that permit a detectable level of binding of the first polypeptide to the second polypeptide in the absence of candidate agent;

(b) determining a level of binding of the first polypeptide to the second polypeptide; and

(c) comparing the level of binding to a control level of binding of the first polypeptide to the second polypeptide in the absence of candidate agent, and therefrom determining whether the candidate agent modulates TGF-β- and/or BMP-mediated signaling.

2. A method according to claim 1, wherein the HECT E3 ubiquitin ligase WW domain comprises the sequence

GPLPXGWEX$_3$tttGtXYYhXHNTtTTtWXtPt (SEQ ID NO:2)

wherein each t is an independently selected polar amino acid residue (*e.g.*, S, H, P, D, E, T or Y), h is a hydrophobic residue (*e.g.*, I, V, L or M) and each X is an independently selected amino acid residue.

3. A method according to claim 1, wherein the Smad PY motif comprises the sequence Ser/Thr-Pro-Pro-Pro-Pro/Ala/Gly-Tyr (SEQ ID NO:15), wherein Ser/Thr is an amino acid residue that is serine or threonine and Pro/Ala/Gly is an amino acid residue that is selected from the group consisting of proline, alanine and glycine.

4. A method according to claim 3, wherein the Smad PY motif comprises the sequence TPPPAY (SEQ ID NO:16) or TPPPGY (SEQ ID NO:18).

5. A method according to claim 1, wherein the candidate agent is a small molecule within a combinatorial library.

6. A method according to claim 1, wherein the first polypeptide is immobilized on a solid support and the second polypeptide comprises a tag.

7. A method according to claim 1, wherein the second polypeptide is immobilized on a solid support and the first polypeptide comprises a tag.

8. A method according to claim 6 or claim 7, wherein the tag is biotin or a radioactive group.

9. A method according to claim 1, wherein the level of binding is determined via a two-antibody sandwich assay.

10. A method according to claim 1, wherein the level of binding is determined via a competitive assay.

11. A method for screening for an agent that modulates TGF-β- and/or BMP-mediated signaling, comprising the steps of:

(a) contacting

(i) a candidate agent;

(ii) a ubiquitinated HECT E3 ubiquitin ligase; and

(iii) a Smad protein or a variant thereof that comprises a PY motif; wherein the contact takes place under conditions and for a time sufficient to permit ubiquitination of the Smad protein or variant thereof by the HECT E3 ubiquitin ligase in the absence of candidate agent;

(b) determining a level of ubiquitination of the Smad protein or variant thereof; and

(c) comparing the level of ubiquitination to a control level of ubiquitination in the absence of candidate agent, and therefrom determining whether the candidate agent modulates TGF-β- and/or BMP-mediated signaling.

12. A method according to claim 11, wherein the method comprises a coupled ubiquitination assay.

13. A method according to claim 11, wherein the ubiquitinated HECT E3 ubiquitin ligase is present within a cell extract fraction.

14. A method according to claim 11, wherein the level of ubiquitination is determined by Western blot analysis.

15. A method according to claim 11, wherein the Smad protein or variant thereof comprises a tag.

16. A method for screening for an agent that modulates BMP-mediated signaling, comprising the steps of:

(a) contacting a cell that expresses a BMP receptor with a bone morphogenic protein and a candidate agent; and

(b) detecting a level of a Smad protein in the cell, relative to a level of the Smad protein in a cell that is contacted with the bone morphogenic protein in the

absence of the candidate agent, and therefrom determining whether the candidate agent is a modulator of BMP-mediated signaling.

17. A method according to claim 16, wherein the Smad protein is Smad1 or Smad5.

18. A method according to claim 16, wherein the cell is a bone cell.

19. A method according to claim 16, wherein the cell is a neuron or kidney cell.

20. A method according to claim 16, wherein the agent enhances BMP-mediated signaling.

21. A method for screening for an agent that modulates BMP-mediated signaling, comprising the steps of:

(a) contacting a cell that expresses a BMP receptor with a bone morphogenic protein and a candidate agent; and

(b) detecting a level of ubiquitination of a Smad protein in the cell, relative to a level of the Smad protein ubiquitination in a cell that is contacted with the bone morphogenic protein but is not contacted with the candidate agent, and therefrom determining whether the candidate agent modulates BMP-mediated signaling.

22. A method according to claim 21, wherein the Smad protein is Smad1 or Smad5.

23. A method according to claim 21 wherein the cell is a bone cell.

24. A method according to claim 21, wherein the cell is a neuron or kidney cell.

25. A method according to claim 21, wherein the agent enhances BMP-mediated signaling.

26. A method for screening for an agent that modulates TGF-β-mediated signaling, comprising the steps of:

(a) contacting a cell that expresses a TGF-β receptor with TGF-β and a candidate agent; and

(b) detecting a level of a Smad protein in the cell, relative to a level of the Smad protein in a cell that is contacted with the bone morphogenic protein in the absence of the candidate agent, and therefrom determining whether the candidate agent is a modulator of TGF-β-mediated signaling.

27. A method according to claim 26, wherein the Smad protein is Smad2 or Smad3.

28. A method according to claim 26, wherein the agent enhances TGF-β-mediated signaling.

29. A method for screening for an agent that modulates TGF-β-mediated signaling, comprising the steps of:

(a) contacting a cell that expresses a TGF-βreceptor with TGF-β and a candidate agent; and

(b) detecting a level of ubiquitination of a Smad protein in the cell, relative to a level of the Smad protein ubiquitination in a cell that is contacted with the bone morphogenic protein but is not contacted with the candidate agent, and therefrom determining whether the candidate agent modulates TGF-β-mediated signaling.

30. A method according to claim 29, wherein the Smad protein is Smad2 or Smad3.

31. A method according to claim 29, wherein the agent enhances TGF-β-mediated signaling.

32. A method for screening for an agent that modulates BMP-mediated signaling, comprising the steps of:

(a) contacting a cell that expresses a BMP receptor with bone morphogenic protein and a candidate agent; and

(b) detecting a level of a HECT E3 ubiquitin ligase activity in the cell, relative to a level of HECT E3 ubiquitin ligase activity in a cell that is contacted with the bone morphogenic protein in the absence of the candidate agent, and therefrom determining whether the candidate agent modulates BMP-mediated signaling.

33. A method according to claim 32, wherein the cell is a bone cell.

34. A method according to claim 32, wherein the cell is a neuron or kidney cell.

35. A method according to claim 32, wherein the agent enhances BMP-mediated signaling.

36. A method for screening for an agent that modulates TGF-β-mediated signaling, comprising the steps of:

(a) contacting a cell that expresses a TGF-β receptor with TGF-β and a candidate agent; and

(b) detecting a level of a HECT E3 ubiquitin ligase activity in the cell, relative to a level of HECT E3 ubiquitin ligase activity in a cell that is contacted with the bone morphogenic protein in the absence of the candidate agent, and therefrom determining whether the candidate agent modulates TGF-β-mediated signaling.

37. A method according to claim 36, wherein the agent enhances TGF-β-mediated signaling.

38. A method for augmenting TGF-β- and/or BMP-mediated signaling in a cell, comprising contacting a cell with an agent that inhibits binding of a HECT E3 ubiquitin ligase WW domain to a Smad PY motif.

39. A method according to claim 38, wherein the Smad PY motif comprises the sequence TPPPAY (SEQ ID NO:16).

40. A method according to claim 38, wherein the Smad PY motif comprises the sequence TPPPGY (SEQ ID NO:18).

41. A method for augmenting TGF-β and/or BMP-mediated signaling in a cell, comprising contacting a cell with an agent that inhibits ubiquitination of a Smad protein.

42. A method according to claim 41, wherein the Smad protein is Smad1 or Smad5.

43. A method according to claim 41, wherein the Smad protein is Smad2 or Smad3.

44. A method for stimulating bone formation in a patient, comprising administering to a patient a therapeutically effective amount of an agent that inhibits binding of a HECT E3 ubiquitin ligase WW domain to a Smad PY motif.

45. A method according to claim 44, wherein the Smad PY motif comprises the sequence TPPPAY (SEQ ID NO:16).

46. A method for stimulating bone formation in a patient, comprising administering to a patient a therapeutically effective amount of an agent that inhibits ubiquitination of a Smad protein.

47. A method according to claim 46, wherein the Smad protein is Smad1 or Smad5.

48. A method for preventing or treating a condition associated with insufficient TGF-β and/or BMP-mediated cell signaling, comprising administering to a patient a therapeutically effective amount of an agent that inhibits binding of a HECT E3 ubiquitin ligase WW domain to a Smad PY motif.

49. A method according to claim 48, wherein the Smad PY motif comprises the sequence TPPPAY (SEQ ID NO:16).

50. A method according to claim 48, wherein the Smad PY motif comprises the sequence TPPPGY (SEQ ID NO:17).

51. A method for preventing or treating a condition associated with insufficient TGF-β and/or BMP-mediated cell signaling, comprising administering to a patient a therapeutically effective amount of an agent that inhibits ubiquitination of a Smad protein.

52. A method according to claim 51, wherein the Smad protein is Smad1 or Smad5.

53. A method according to claim 51, wherein the Smad protein is Smad2 or Smad3.

Rl.126 54. ~~53.~~ A method according to claim 48 or claim 51, wherein the condition is a cancer or inflammation.